Environmental Health Perspectives
Vol. 35, pp. 39-42, 1980

Bronchus-Associated Lymphoid Tissue and the Source of Immunoglobulin-Containing Cells in the Mucosa

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Bronchus-associated lymphoid tissue has major morphologic and functional similarities to Peyer's patches found in the gut. Both possess a lymphoepithelium with selective antigen sampling properties, both appear in the apparent absence of direct antigen stimulation, both contain a high percentage of cells bearing IgA surface immunoglobulin and both can repopulate the bronchial and gut lamina propria with IgA containing cells.

Good evidence now exists (and will be reviewed) in support of the concept of a common mucosal immunologic system. Cells potentially sensitized at or in a mucosal tissue such as the gut or lung would then migrate to the draining lymph node, thence into the circulation and localize in a variety of mucosal tissues. Factors involved but not essential for such localization include antigen. Lymphoblasts derived from the lung tend to go back to the lung. Similarly, gut derived lymphoblasts have a predilection for the gut. However, available evidence supports the concept of integrated systemic and mucosal immune systems.

Several factors must be taken into account in analysis of the products of local mucosal immune reactions and in developing approaches to achieve optimal humoral immunity at any mucosal surface.

Although the lungs are not usually regarded as primary lymphoid organs, it may be of interest to recall that in 1958 Humphrey and co-workers (*1, 2*) showed that following intravenous hyperimmunization with pneumococcal antigens, the lung tissue was the predominant source of specific antibodies surpassing even the bone marrow, spleen and lymph nodes. One explanation for these findings may have been that the lungs contained a substantial population of immunocompetent cells present by either direct immigration from the circulation or by local proliferation of a precursor population. Also, it appears from this work that the lungs can respond well to antigens introduced parenterally.

A few years ago we observed the presence of follicular lymphoid aggregates in the mucosa of the rabbit bronchial tract with a peculiar epithelial relationship (*3, 4*). These lymphoid follicles can, with practice, be discerned as dense white patches under the dissecting microscope or upon treatment of the respiratory tract with 2% acetic acid. The distribution of this lymphoid tissue is random along the length of the bronchial tract as far as the small bronchioles and seems to be concentrated around bifurcations. The tissue is present in all mammals examined so far with the possible exception of the Golden hamster. Chickens also possess considerable amounts of this tissue which project into the lumens of the large bronchi. Morphologically, this bronchus-associated lymphoid tissue (BALT) was remarkably similar to the Peyer's patches of the gut. Interestingly, Klein in 1875 (*5*) wrote, ". . . these lymphoid follicles of the bronchial walls are, therefore, in every respect analogous to the lymph follicles found in other mucous membranes, e.g. tonsils and in the intestine."

The epithelium which overlies the BALT follicles is unlike that found elsewhere in the bronchus and is constructed of flattened, irregular shaped cells. Often the BALT epithelium is heavily infiltrated with lymphocytes from the underlying follicles (*6*). This lymphoepithelium is reminiscent of the follicle-associated epithelium overlying Peyer's patches in the gut (*7*) and is deficient in glandular tissue and goblet cells as well. In addition, cilia are irregularly

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distributed and ultrastructural examination reveals the presence of microvilli. Rare plasma cells are seen to be scattered throughout the lymphoid follicles and occasionally plasma cells are found in the overlying lymphoepithelium (*8*). The follicles contain the highwalled endothelium associated with post-capillary venules and this type of endothelium appears to be more prominent following antigen challenge (*8*).

The lymphoepithelium seems to act as a sampler of the lumenal antigen much as does the follicle-associated epithelium of the Peyer's patches and also the bursa of Fabricius (*7*). Horseradish peroxidase introduced into the lumen of the rabbit tracheobronchial tree is selectively taken up by the lymphoepithelium overlying the BALT follicles (*9*). A fairly extensive proliferation of cells in the follicles occurs after antigen challenge, particularly with *Bacillus Calumet-Guerin* (*8*), and following *in vivo* injections of tritiated thymidine, heavily labeled cells are seen 24 hr later in a crescentric manner capping the lumenal aspects of the follicles (*4*). However, the BALT seems to develop in the virtual absence of antigen. When fetal pulmonary tissue was transplanted to ectopic subcutaneous sites in syngeneic adult mice, BALT follicles still developed but appeared immature (*4, 10*). The fact that germ-free animals possess a BALT mass considerably less than that seen in conventional animals strongly suggests that antigen promoted complete BALT development (*3*).

The predominant type of cells present in BALT, as judged by surface membrane antigens, are neither T nor B cells (*11*). In young adult rabbits, 18.4% of BALT cells bore a thymic antigen whereas in Peyer's patches and intestinal lamina propria, this figure was 16.6% and 11.1% respectively. Approximately 40% of BALT cells bore surface immunoglobulin and 17.2% of these had surface IgA, a value intermediate between that of Peyer's patches (29.9%) and gut lamina propria (7.3%). The finding of BALT intermediacy between the Peyer's patches and the intestinal lamina propria provides some clues as to the functions of the BALT.

Some evidence indicates that the BALT is functionally similar to the Peyer's patches. It has been demonstrated (*12*) that cells derived from the BALT had virtually the same capacity as Peyer's patch cells to repopulate the spleen of irradiated allogeneic recipients with IgA-containing cells. Of greater significance, cells derived from the BALT were nearly as effective as Peyer's patch cells in repopulating the lamina propria of the bronchus and gut with IgA plasma cells. These findings demonstrated that cells from one mucosal site could move, and perhaps differentiate at another mucosal site and supported our original hypothesis, in 1973, that the BALT might be part of a more universal mucosal lymphoid system (*3*).

In 1974 we proposed that there may be a common mucosal immunologic system (*13*). Montgomery (*14*) has shown that rabbits orally immunized with DNP pneumococci have a selective appearance of specific IgA antibody in the milk in the apparent absence of such antibody in the circulation. Similar feeding experiments have been done by Hanson and co-workers in humans using a nonpathogenic *E. coli* (15). The subjects which had received *E. coli* subsequently developed a rise of specific IgA antibody against *E. coli* in the milk and antigen specific IgA antibody-containing cells appeared in their colostrum. Lamm and co-workers (*16*) have shown that dividing cells from the mesenteric lymph node have a tendency to selectively localize in the mammary glands when adoptively transferred into syngeneic mice in late gestation. This selective migration was enhanced greatly by lactation and could be induced in nonpregnant animals by the suitable injection of sex hormones. Thus, the antibody and cells in milk specific for enteric antigens probably results from the migration of cells from gut to breast tissue. We have done similar experiments with dividing cells from the bronchial lymph nodes and mesenteric lymph nodes and have shown that these cells have a tendency to home to mucosal tissues including the breast, bronchus, bowel and cervix where they make predominantly IgA (*17*). Thus, it seems likely that a common mucosal system may well exist, at least for those mucosal sites where cells are potentially primed or sensitized and have a tendency to traffic to another mucosal site.

One question which has not been completely answered is what the role of antigen might be in lymphocyte traffic between mucosal surfaces. This traffic appears to be independent of antigen although the presence of antigen, to which the cells have been primed, clearly causes a greater number of IgA cells to appear in the lamina propria of the bowel mucosa (*18*). It may be postulated that antigen might cause cells to divide locally or even retain cells once they have immigrated into the mucosal lamina propria. In this event, if cells were not exposed to the sensitizing antigen they might leave (presumably by lymphatics), die, or alternatively, find their way via the mucosal epithelium into the lumenal spaces. The cytokinetic experiments referred to earlier found heavily labelled cells within the lymphoepithelium of the BALT 24 hr after the *in vivo* injection of tritiated thymidine (*4*). We have chosen to interpret this finding as partial support for the suggestion that these cells may well be on their way out into the lumen although no direct evidence for this exists.

Careful examination of preparations of BALT led

to the appreciation that there were a number of cells present which had metachromatic granules in the cytoplasm giving the appearance of primitive mast cells (*6*). Since some of these cells may well have emanated from the bronchial lamina propria, a systematic attempt was initiated to look for them in BALT tissue. We did indeed find, with special stains and using ultrastructural techniques, the presence of basophil-like cells, particularly in the peripheral zones of the BALT follicles. These cells were also seen above the basement membrane in the epithelium adjacent to these follicles and resembled the basophiloid cells which have been described by Patterson in several animal species in the bronchial washings (*19*). Collan (*20*) has described granular lymphocytes in the rat intestinal epithelium. The relationship of these cells to mast cells is unclear but we have also observed such granular lymphocytelike cells in preparations from the gut lamina propria and have identified these primarily in the epithelium in rabbits (*21*). Recently, Guy-Grand et al. (*22*) have made a number of observations about these cells in the small intestine and have concluded that the cells in the gut epithelium appear to arise from a special population of T cells present in the Peyer's patches. It is tempting to speculate that a similar Peyer's patch (or BALT?) derivation applies to the basophiloid cells in bronchial epithelium. Metachromatic, granule-containing cells are also found in the Peyer's patches. These cells, which lie in the spaces formed between epithelial cells, are potentially in direct contact with antigen which traverses the mucosal epithelium and is then exocytosed into the lateral inter-epithelial spaces. Much more work needs to be done in this area to establish the relationship of epithelial basophiloid cells to T cells or mast cells and to explore the functional activity of this cell type.

There is some evidence for T blast traffic between mucosal surfaces of a similar nature to that described above for B blasts, particularly precursors of IgA (*23*). Relatively little is known about T blast traffic to the respiratory tract and it is an open question whether or not such a common mucosal system exists for T cells. Waldman and Henney (*24*) first showed that following intratracheal immunization, cells washed out from the lungs could proliferate and release migration inhibition factor (MIF) on exposure to specific antigen. It is unclear whether the cells from which this lymphokine were derived were T cells or B cells especially since both types of cells will produce MIF. In any event, local immunization has been shown to produce a local cell mediated specific immune reaction. Immunization protocols have been devised mostly in experimental animal models to prove that local immunization results in local antibody and local cell mediated immune reactions; such reactions are more consistent and of a higher intensity than when antigen is introduced parenterally. It must be remembered when looking at these results that the lung, from the standpoint of mucosal immune responses, is divided into at least two compartments, that which is mucosal and that which is peripheral and very similar to the blood in terms of the antibodies contained therein (*25*). If one draws an analogy with the bowel, it appears especially from the work of Pierce and colleagues (*18, 26*) that parenteral immunization followed by local challenge leads to a higher accumulation of IgA antibody-producing cells in the intestine than with any other immunization protocol. It follows, therefore, that antibody and concomitant protection against a particular organism might best be achieved locally in the respiratory tract if parenteral immunization occurred first and was then followed by local presentation of antigen. The best way to prime for complete protection of the lungs against subsequent infection is ill understood.

Local immunization of the lungs may lead to local IgE antibody formation which could, of course, be deleterious. Gerbrandy and Bienenstock (*27*) have shown that, following intratracheal immunization, potential IgE-forming cells could be demonstrated in the draining mediastinal lymph nodes in greater numbers than following peripheral immunization. Intraperitoneal immunization also produced the same results but this observation is confusing since it has been known for many years that intraperitoneal immunization is a good method of priming for the IgE response and antigens introduced into the peritoneal cavity will drain through the diaphragm into the mediastinal nodes. Obviously, until the potential hazards of producing a local IgE type of immune response versus a protective immune response at a local mucosal surface are better understood, immunization protocols will perforce not be able to maximize and mobilize the appropriate immune response at the appropriate mucosal site.

This work was supported by the Medical Research Council of Canada.

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